Chapter 6

Hemodialysis Induces an Acute Decline in Cerebral Blood Flow in Elderly Patients

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ABSTRACT

The initiation of hemodialysis is associated with an accelerated decline of cognitive function and an increased incidence of cerebrovascular accidents and white matter lesions. Investigators have hypothesized that the repetitive circulatory stress of hemodialysis induces ischemic cerebral injury, but the mechanism is unclear. We studied the acute effect of conventional hemodialysis on cerebral blood flow (CBF), measured by $[^{15}O]H_2O$ positron emission tomography–computed tomography (PET-CT). During a single hemodialysis session, three $[^{15}O]H_2O$ PET-CT scans were performed: before, early after the start of, and at the end of hemodialysis. We used linear mixed models to study global and regional CBF change during hemodialysis. Twelve patients aged ≥65 years (five women, seven men), with a median dialysis vintage of 46 months, completed the study. Mean (±SD) arterial BP declined from 101±11 mmHg before hemodialysis to 93±17 mmHg at the end of hemodialysis. From before the start to the end of hemodialysis, global CBF declined significantly by 10% ±15%, from a mean of 34.5 mL/100 g per minute to 30.5 mL/100 g per minute (difference, -4.1 mL/100 g per minute; 95% confidence interval, -7.3 to -0.9 mL/100 g per minute; *P*=0.03). CBF decline (-20%) was symptomatic in one patient. Regional CBF declined in all volumes of interest, including the frontal, parietal, temporal, and occipital lobes; cerebellum; and thalamus. Higher tympanic temperature, ultrafiltration volume, ultrafiltration rate, and pH significantly associated with lower CBF. Thus, conventional hemodialysis induces a significant reduction in global and regional CBF in elderly patients. Repetitive intradialytic decreases in CBF may be one mechanism by which hemodialysis induces cerebral ischemic injury.

INTRODUCTION

More than 2 million individuals with ESRD worldwide receive RRT, of which hemodialysis (HD) is the most frequently used modality. [1, 2] Especially in elderly patients receiving HD cognitive impairment is highly common, with a prevalence up to 60%. [3-5] Decline of cognitive function, especially of executive function, is already present in patients with mild to moderate CKD and the transition to dialysis is associated with a significant loss of executive function. [6-10]

There is increasing evidence that the HD procedure itself might contribute to brain injury. First, it was reported that stroke incidence rose in the first month of HD in elderly patients and remained elevated afterward compared with the period before initiation of HD. [11] Second, a longer HD vintage is associated with reduced white matter integrity on magnetic resonance imaging (MRI). [12-14] Finally, lowering the dialysate temperature resulted in an improvement in intradialytic hemodynamic stability and strongly attenuated the progression of white matter lesions during the first year of HD, providing indirect evidence that the HD procedure contributes to cerebral ischemia. [15] At present, the mechanism by which HD could contribute to brain damage is unknown. For the heart, it was shown that HD induces a fall in myocardial blood flow resulting in subclinical myocardial ischemia. [16-19] Likewise, we hypothesized that a repetitive HD-induced cerebral blood flow (CBF) decline may lead to (cumulative) ischemic brain lesions. These lesions may contribute to the accelerated cognitive decline after the initiation of HD. To our knowledge, no study has yet evaluated the acute effect of HD on CBF using quantitative CBF measurements. We aimed to study the effect of HD on CBF early and late during the dialysis procedure using $[^{15}O]H_2O$ positron emission tomography–computed tomography (PET-CT) scans, which are considered the gold standard for CBF measurement. [20-22] The primary objective was to evaluate the effect of HD on global and regional CBF. The secondary objective was to explore associations of HD treatment-related factors with CBF.

CONCISE METHODS

Patients and Study Design

This study was performed according to the principles of the Declaration of Helsinki and was approved by the Medical Ethical Committee of the University Medical Center Groningen, and registered at clinicaltrials.gov (NCT02272985). All patients gave written informed consent. The study was performed between March and November of 2015.

Patients receiving HD aged ≥65 years from our department with an arteriovenous fistula without significant recirculation were eligible for this study. Patients were studied during a regular dialysis session after the longest interdialytic interval (Monday or Tuesday). Patient

characteristics were assessed at study entry and retrieved from the patients' medical history. Height was measured before, and weight before and after the PET-HD session. BP, heart rate, and tympanic temperature were measured before every PET-CT scan and every 30 minutes during the HD study session. For more information on study design, including additional in- and exclusion criteria, we refer to the Full Concise Methods (Supplemental Material).

HD Study Session

All HD study sessions were performed in the afternoon in the PET-camera room. The ambient temperature of the room was kept constant at 20˚C, excluding an effect of outside temperature on cardiovascular stability during study sessions. After the first PET scan (T1), patients started dialysis still being in a horizontal position in the PET-camera. After the second PET scan (T2), which was performed within 30 minutes after the start of HD, patients were transferred to a hospital bed adjacent to the PET-camera to continue dialysis in a 30-45-degree supine position. Approximately 30 minutes before the start of the third PET scan (T3), which was performed in the final hour of the HD session, patients were transferred back to the PET.

A low-dose brain computed tomography was made before the first and third PET-scan to correct for attenuation of the PET data. A bolus injection of $[^{15}O]H_2O$ was administered intravenously at a constant rate through an indwelling peripheral venous catheter in the non-dialysis access arm. The injected dose of $[^{15}O]H_2O$ was 500 MBq per scan, with a total dose of 1500 MBq per patient for the whole study. During each PET-scan, arterial blood was sampled continuously from the dialysis line by a dedicated programmable blood-sampler to obtain the course of the radioactivity concentration in the blood during 5 minutes following the injection of $[^{15}O]H_2O$. To perform laboratory measurements, arterial blood was sampled from the arterial dialysis line just before each PET-scan.

Dialysis Settings

All patients were on bicarbonate dialysis with a low-flux polysulfone hollow-fiber dialyzer (F8; Fresenius Medical Care, Bad Homburg, Germany). Blood flow and dialysate flow rates were 200-300 and 500 mL/min, respectively. Dialysate temperature was 36.5°C in all patients. We used constant UF rate and dialysate conductivity. For dialysate composition we refer to the Full Concise Methods (Supplemental Material).

PET Data Acquisition

For the $[^{15}O]H_2O$ PET-CT scans a Siemens Biograph 64-mCT (Siemens Medical Systems, TN) that acquires 109 planes over a total axial length of 216 mm was used. For details on the $[^{15}O]H_2O$ production we refer to the Full Concise Methods (Supplemental Material).

First, a low-dose computed tomography scan was performed for attenuation and scatter correction. The dynamic PET acquisition (310 seconds) was started, followed after

10 seconds by an intravenous bolus injection of [^{15}O]H_2O. In total, the duration of every PET-CT scan was 5 minutes, which was uniform across all time points and all patients. Head movement was minimized with a head-restraining band. For CBF quantification, the arterial input function was obtained from arterial blood radioactivity, which was continuously monitored with an automated sampling system (Veenstra Instruments, Joure, the Netherlands). One extra blood sample was collected at 393±32 seconds after tracer injection to determine the amount of radioactivity in the blood using a γ-counter (Wizard2, Perkin Elmer, Waltham).

Three of the 36 scans could not be analyzed due to a technical problem with the automated sampling system during the measurement (patient identity 106 [T1], patient identity 107 [T2], patient- identity 102 [T3]).

MRI Data Acquisition

MRI was performed using a 1.5T whole body system (Aera, Siemens, Erlangen, Germany) on a non-dialysis day. The study MRI was performed median 3 days (range, -72 to +3 days) after the HD study session. The scan protocol (total scan time 30 minutes) included T1-weighted, T2-weighted, three-dimensional fluid-attenuated inversion recovery, diffusion-weighted imaging, susceptibility weighted imaging, and two-dimensional phase contrast sequences. No intravenous contrast was used. A neuroradiologist (PJvL) assessed white matter hyperintensities, and cortical atrophy, using the Fazekas scale and the global cortical atrophy scale, respectively. [44, 45] Microbleeds were scored on the susceptibility weighted imaging sequence.

Image Reconstruction and Preprocessing

Image processing and pharmacokinetic analysis were performed with PMOD 3.8 software (PMOD Technologies Ltd., Zurich, Switzerland). The average image (time-weighted) was used for rigid matching registration of the individual PET to the individual MRI. See Full Concise Methods (Supplemental Material) for background information on image reconstruction and processing.

Neuropsychological Tests

A neuropsychological assessment battery was performed to characterize the study population and included all major cognitive domains. For details on the neuropsychological assessment battery we refer to the Full Concise Methods (Supplemental Material). The order of the tests was fixed and cognitive testing was performed on a nondialysis day. It took approximately 45-60 minutes per subject to complete the tests. The neuropsychological assessment was performed median 95 days (range, -196 to -33 days) before the HD study session.

Statistical Analyses

Intradialytic changes in levels of the HD-related characteristics were studied using repeated measures ANOVA (with a Greenhouse-Geisser correction in case of non-sphericity), with a Bonferroni correction.

For the primary study objective, global and regional CBF changes were analyzed by LMM, which allowed for individual random intercepts and slopes of CBF over time. The random slopes were on the basis of the actual scan times per patient. Relative CBF change was calculated as the mean of the individual percentual change between T1 and T3 using descriptive statistics, and is reported as mean±SD (%).

For the secondary study objective, associations of HD treatment-related factors, which might potentially explain CBF change, with CBF were studied. Those factors included MAP, pCO_2, pH, tympanic temperature, hematocrit, and UF volume and were selected based on literature. [15, 23, 24, 26, 34, 46-48] The factors were studied univariately using LMM, checking the significance of interactions with scan-order. Because UF volume was associated with CBF, the association between UF rate and CBF was evaluated as well.

In additional analyses, associations of cognitive test scores and structural brain characteristics with CBF were explored. To this end, we first tested correlations with baseline CBF using Pearson or Spearman correlation, if appropriate. Subsequently, we studied the associations including all CBF measurements in an LMM. For these analyses, the cognitive test scores were converted to Z scores.

Several sensitivity analyses were performed. First, regional CBF change was also calculated for the left and right hemisphere separately. Second, in order to eliminate a possible effect of HD on the arterial sampling from the arteriovenous fistula, CBF change between T2 and T3 was calculated. Third, CBF change in only the gray matter of each VOI was studied instead of the sum of gray and white matter of the corresponding region.

Two-sided $P<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS, version 23 (SPSS Inc., IBM company), GraphPad Prism version 5.0 (GraphPad Software, San Diego), and R version 3.4.0 (R Core Team, 2017).

RESULTS

Enrolment and Patient Characteristics

Of 78 eligible patients aged ≥65 years, 64 patients were asked to participate, and 15 patients gave written informed consent (Figure 1). None of the patients had to be excluded because of a significant carotid artery stenosis. Three patients withdrew from the study, because of a kidney transplantation, hip fracture, and withdrawal of consent, respectively. Twelve patients completed the study, of whom the characteristics are summarized in Table 1.

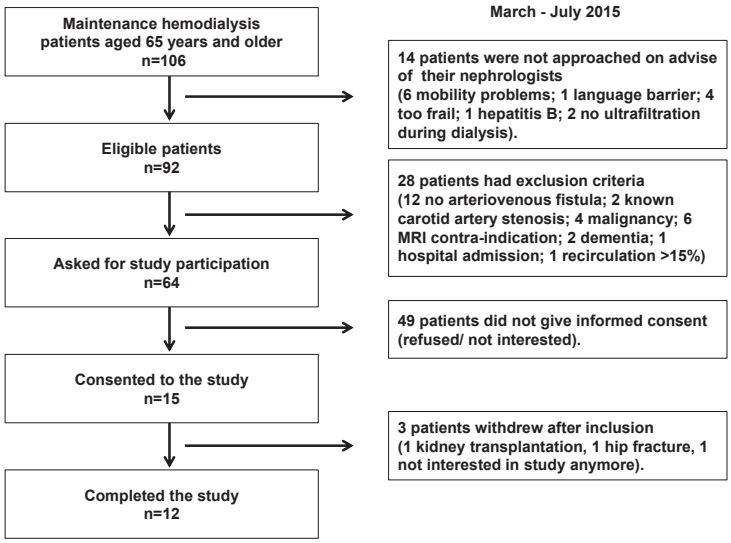

Figure 1 Study flow chart demonstrating the phases of the study starting from screening through to inclusion and completion of the study.

Table 1 Patient characteristics

Characteristic	Total (*N*=12)
Age	75.4 ± 5.2
Male sex	7 (58%)
BMI (kg/m^2)	26.6 ± 3.5
Primary kidney disease	
Glomerulonephritis	4 (33%)
Diabetes	1 (8%)
Vascular	3 (25%)
Other diagnosis	3 (25%)
Unknown	1 (8%)
Dialysis vintage (months)	46 (range 11-319)
Dialysis treatment time (hours per week)	12 (range 8-15)
Kt/V (per week)	3.91 ± 0.73
% IDH-complicated HD sessions 30 days prior to study session [a]	
Never	8 (67%)
In 10 to 20% of HD sessions	2 (17%)
In 30 to 40% of HD sessions	2 (17%)
Comorbidities	
Diabetes	3 (25%)
Hypertension	11 (73%)
Myocardial infarction	2 (17%)
Heart failure	1 (8%)

Table 1 Patient characteristics (*continued*)

Characteristic	Total (*N*=12)
Peripheral artery disease	1 (8%)
COPD	1 (8%)
Depression	1 (8%)
Medication	
CCB	4 (33%)
Nitrate	3 (25%)
ACE inhibitor	1 (8%)
Angiotensin receptor blocker	1 (8%)
B-blocker	9 (75%)
Neuropsychological assessment	
MMSE [b]	28 (range 25-29)
RAVLT, delayed recall [c]	6.8 ± 3.4
Digit span forward [c]	5.1 ± 0.8
Digit span backward [c]	3.8 ± 1.1
TMT-A (sec) [c]	71.3 ± 28.6
TMT-B (sec) [c]	200 ± 94
TMT B/A ratio [d]	2.59 ± 0.90
Letter fluency [c]	24.6 ± 12.1
Clock drawing score [c]	14 (range 9-14)
HADS depression score [d]	6.0 ± 3.9
HADS anxiety score	3.9 ± 3.6
MRI brain	
GCA score	
0 - no atrophy	1 (8%)
1 - mild atrophy	8 (67%)
2 - moderate atrophy	3 (25)
3 - severe atrophy	0
Fazekas score of WML	
0 - no WML	1 (8%)
1 - multiple punctate lesions	4 (33%)
2 - beginning confluent lesions	5 (42%)
3 - large confluent WML	2 (17%)
Microbleeds	7 (58%)

Data are presented as mean ± SD or median (range), or percentages (%). BMI, body mass index; IDH, intradialytic hypotension; COPD, chronic obstructive pulmonary disease; CCB, calcium channel blocker; ACE, angiotensin-converting enzyme; MMSE, Mini Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test; TMT, trail making test; HADS, hospital anxiety depression scale; GCA, global cortical atrophy; WML, white matter lesions.

[a] IDH was defined as an SBP drop <100 mHg, any IDH-related intervention during HD, or IDH symptoms including dizziness or loss of consciousness. [b] An MMSE score ≥24 indicates normal cognition. [c] The number of patients that were impaired according the age-, sex-, and education-adjusted norm scores were: 0 (RAVLT), 2 (digit span, only age-adjusted), 4 (TMT-A, TMT-B), 2 (letter fluency), and 1 (clock drawing). [d] Three patients had a TMT B/A ratio >3.0 indicating executive function impairment. [e] A score >7 on the HADS depression (*n*=3) or anxiety (*n*=1) indicates the presence of symptoms of depression or anxiety, respectively.

HD Study Session Characteristics

During a single HD session three [^{15}O]H_2O PET-CT scans were performed: Before (T1), shortly after the start of HD (T2), and at the end of HD (T3). The mean time interval between T1 and T2 was 39 minutes (range 28-61 minutes). The second and third scans were performed at a mean of 21 minutes (range, 13-29 minutes) and 209 minutes (range 168-223 minutes) after the start of HD, respectively. Intradialytic changes in vital and laboratory parameters are shown in Table 2. Mean ultrafiltration (UF) volume was 1934±781 mL, UF rate 6.7±2.5 mL/h per kilogram, and weight change -1.6±0.7 kg.

Table 2 Intradialytic changes in vital and laboratory values

	Before start HD	After start HD	At the end of HD	Dialysis treatment effect	
	T1	T2	T3	T1 vs. T3	T2 vs. T3
SBP (mmHg)	152 ± 22	157 ± 26	140 ± 30	-9 (-27 to 10)	-15 (-36 to 5)
DBP (mmHg)	75 ± 8	78 ± 13	70 ± 12	-5 (-14 to 4)	-7 (-17 to 3)
MAP (mmHg)	101 ± 11	105 ± 15	93 ± 17	-6 (-15 to 3)	-10 (-19 to -0.1) [a]
Heart rate (bpm)	69 ± 9	68 ± 10	72 ± 9	4 (-3 to 11)	5 (1 to 12)
Tympanic temperature	36.3 ± 0.5	36.2 ± 0.5	35.9 ± 0.6	-0.3 (-0.8 to 0.3)	0.1 (-0.3 to 0.6)
Hemoglobin (mmol/L)	6.7 ± 0.8	6.4 ± 0.9	7.1 ± 0.9	0.4 (0.1 to 0.7) [a]	0.7 (-0.4 to 1.0) [b]
Hematocrit (v/v)	0.33 ± 0.04	0.31 ± 0.04	0.34 ± 0.04	0.02 (0.002 to 0.03) [a]	0.03 (0.02 to 0.05) [b]
Glucose (mmol/L)	6.4 ± 1.5	5.7 ± 1.1	7.7 ± 1.1	1.3 (-0.5 to 3.2)	2.0 (0.6 to 3.4) [c]
pO_2 (kPa)	12.2 ± 2.1	11.5 ± 1.8	12.5 ± 2.6	0.4 (-1.4 to 2.2)	1.0 (-0.6 to 2.6)
pCO_2 (kPa)	5.0 ± 0.5	5.2 ± 0.5	5.1 ± 0.5	0.1 (-0.1 to 0.3)	-0.02 (-0.4 to 0.3)
pH	7.38 ± 0.04	7.40 ± 0.03	7.48 ± 0.04	0.10 (0.07 to 0.13) [b]	0.08 (0.05 to 0.11) [b]
Creatinine (umol/L)	798 ± 190	713 ± 176	313 ± 95	-485 (-588 to -382) [b]	-400 (-488 to -312) [b]
Urea (mmol/L)	24.0 ± 6.6	21.7 ± 6.5	8.3 ± 2.3	-15.7 (-20.4 to -11.1) [b]	-13.5 (-18.1 to -8.8) [b]
Sodium (mmol/L)	139 ± 2	139 ± 2	141 ± 2	1.8 (-0.03 to 3.7)	1.3 (-0.6 to 3.2)
Potassium (mmol/L)	5.1 ± 0.9	4.7 ± 1.0	3.4 ± 0.4	-1.6 (-2.4 to -0.9) [b]	-1.3 (-2.0 to -0.6) [c]
Bicarbonate (mmol/L)	22 ± 2	23 ± 2	28 ± 2	6.5 (4.8 to 8.3) [b]	4.9 (3.5 to 6.4) [b]
i-Calcium (mmol/L)	1.18 ± 0.05	1.20 ± 0.06	1.23 ± 0.08	0.05 (0.01 to 0.09) [a]	0.03 (0.005 to 0.06) [a]
Lactate (mmol/L)	1.02 ± 0.36	0.69 ± 0.21	1.37 ± 0.53	0.35 (0.03 to 0.70) [a]	0.68 (0.33 to 1.02) [c]
CRP (mg/L)	7.9 ± 6.3	7.5 ± 6.1	8.8 ± 8.0	0.9 (-1.1 to 2.9)	1.4 (-0.5 to 3.2)
PTX 3 (ng/mL)	1.89 ± 0.83	2.08 ± 1.36	3.62 ± 1.72	1.73 (-2.77 to -0.69) [c]	1.54 (0.98 to 2.09) [b]
MPO	1.00 ± 0.19	1.76 ± 0.69	1.38 ± 0.33	0.38 (0.08 to 0.68) [a]	-0.39 (-0.86 to 0.09)
vWF (%)	158 ± 43	141 ± 45	160 ± 49	0.1 (-22 to 23)	15 (-6 to 37)

Data are presented as unadjusted means ±SD. Dialysis treatment effects are presented as mean differences (95% CI) obtained from repeated measurements ANOVA models. DBP, Diastolic BP; i-Calcium, ionized calcium; CRP, C-reactive protein; PTX 3, Pentraxin 3; MPO, Myeloperoxidase; vWF, van Willebrand Factor. [a] $P<0.05$, adjusted for multiple comparisons by Bonferroni. [b] $P<0.001$, adjusted for multiple comparisons by Bonferroni. [c] $P<0.01$, adjusted for multiple comparisons by Bonferroni.

The Effect of HD on systemic BP and CBF

Mean arterial pressure (MAP) initially increased from 101±11 (T1) to 105±15 (T2) and then decreased significantly to 93±17 mmHg at the end of HD (T3). The lowest individual nadir in systolic blood pressure (SBP) during the HD study session was 105 mmHg. The change in SBP and MAP between the start of HD and the nadir during HD ranged from -46 to +3 mmHg, and from -23.3 to +9.7 mmHg, respectively (Supplemental Table S1).

Global crude CBF levels of the individual patients are shown in Figure 2. On average, global CBF declined from a baseline of 34.5 (31.4-37.9) mL/100 g per minute to 30.5 (27.7-33.3) mL/100 g per minute at the end of HD in the linear mixed models (LMM) analysis (difference, -4.1 mL/100 g per minute; 95% confidence interval [95% CI], -7.3 to -0.9; *P*=0.03) (Table 3). Regionally, CBF declined in all volumes of interest (VOIs) (Figure 3, Table 3).

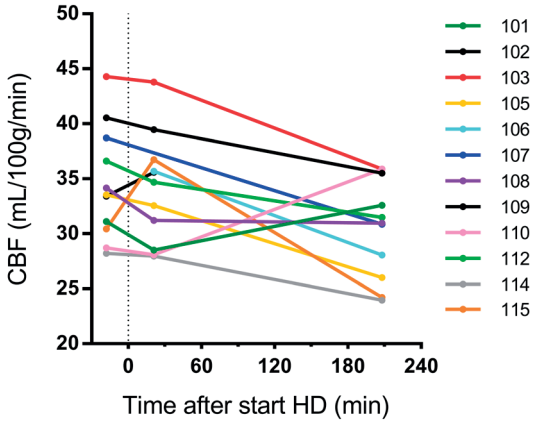

Figure 2 Individual crude CBF trajectories during hemodialysis. Scan 1 was performed at a mean of 18 min (range 15-31 min) before the start of HD. HD is regarded as baseline (t=0). Scan 2 and scan 3 were performed at a mean of 21 minutes (range 13-29 min) and 209 minutes (range 168-223 min) after the start of HD, respectively. Each line represents one patient. In three patients, CBF trajectories are incomplete since 1 scan was missing; identity 102: T3, identity 106: T1, and identity 107: T2.

The relative change in crude CBF between T1 and T3 could be calculated for ten patients. Using descriptive statistics, the average (±SD) change in CBF was -10±15% for global, -11±17% for frontal, -11±16% for parietal, -10±14% for temporal, -9±13% for occipital, -10±13% for cerebellum, and -10±16% for thalamus perfusion.

Table 3 Intradialytic changes in CBF (ml/100 g per minute)

	Before start HD	After start HD [a]	At the end of HD [a]	Dialysis treatment effect [b]	
Brain region	T1	T2	T3	T1 vs. T3	T2 vs. T3
Global	34.5 ± 5.1	34.0 ± 5.0	30.5 ± 4.4	-4.1 (-7.3 to -0.9) [c]	-3.8 (-7.2 to -0.5) [c]
Regional:					
Frontal lobe	40.2 ± 6.9	38.9 ± 5.6	35.0 ± 4.7	-5.1 (-9.5 to -0.6) [c]	-4.1 (-7.8 to -0.3) [c]
Parietal lobe	37.4 ± 7.0	36.3 ± 6.2	32.6 ± 5.1	-4.7 (-8.7 to -0.8) [c]	-4.0 (-7.4 to -0.6) [c]
Temporal lobe	35.8 ± 5.1	35.7 ± 5.6	31.8 ± 4.7	-4.0 (-7.4 to -0.6) [c]	-4.0 (-6.9 to -1.0) [d]
Occipital lobe	41.9 ± 5.1	41.6 ± 5.1	37.7 ± 5.6	-4.4 (-8.4 to -0.3) [c]	-4.5 (-8.1 to -1.0) [d]
Cerebellum	43.3 ± 6.8	44.8 ± 7.4	38.4 ± 6.2	-5.0 (-9.2 to -0.8) [c]	-6.3 (-10.0 to -2.6) [e]
Thalamus	47.3 ± 7.2	48.1 ± 8.4	41.7 ± 8.3	-5.5 (-11.1 to 0.2)	-6.6 (-11.5 to -1.7) [d]

Data are presented as unadjusted mean ± SD.

[a] Scan 2 and 3 were performed at mean 21 and 209 minutes after start of HD, respectively. [b] Dialysis treatment effects are obtained from linear mixed effects models and presented as mean difference (95% CI). [c] $P<0.05$; [d] $P<0.01$; [e] $P<0.001$.

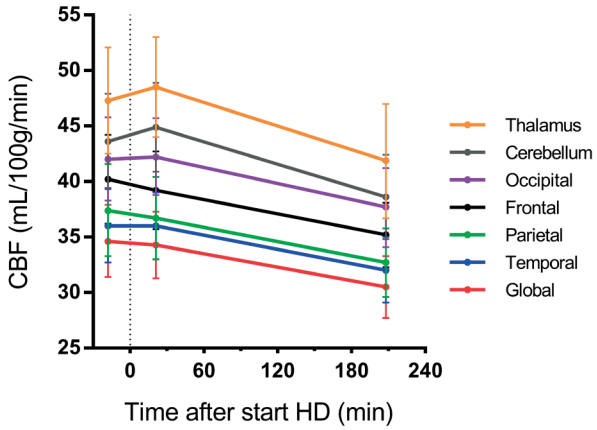

Figure 3 Global and regional CBF declined during HD. The CBF trajectories are shown with 95% CI's (vertical lines), and were calculated from squares means according to linear mixed models. Scan 1 was performed at a mean of 18 min (range 15-31 min) before the start of HD. HD is regarded as baseline (t=0). Scan 2 and scan 3 were performed at a mean of 21 minutes (range 13-29 min) and 209 minutes (range 168-223 min) after the start of HD, respectively.

Associations of HD Treatment-Related Factors with CBF

To investigate the secondary objective, we explored *a priori* selected HD treatment-related factors that might potentially explain an intradialytic CBF change, using LMM. A higher UF volume, a higher tympanic temperature, and a lower pCO_2 were associated with a lower CBF in almost all VOIs (Table 4). A higher UF rate was associated with lower frontal and temporal CBF (estimated effect, -1.2 mL/100 g per minute; 95% CI, -2.1;

-0.1; *P*=0.03 on frontal CBF; and -1.2 mL/100 g per minute; 95% CI -2.0; -0.3; *P*=0.02 on temporal CBF). A significant interaction of pH with scan-order was present for the association between pH and CBF in almost all VOIs. Higher pH was significantly associated with a lower regional CBF at T2 as compared with T1, but not at T3, except for frontal CBF (estimated interaction effect pH*T3, -27.4 ml/100 g per minute; 95% CI, -44.9 to -3.8; *P*<0.001). Hematocrit was only associated with CBF in one VOI. When pH, UF volume, or tympanic temperature were added to the model, the effect of scan-order became nonsignificant. The analysis of MAP and CBF was limited by insufficient power due to considerable patient variation in MAP.

Table 4 Associations of *a priori* selected HD treatment-related factors with CBF

	pCO_2 (kPa)	pH (per 0.1 change)		Temperature (°C)	UF volume (L)
Region	Estimated effect on CBF (ml/100 g per min)	Estimated effect on CBF (ml/100 g per min)		Estimated effect on CBF (ml/100 g per min)	Estimated effect on CBF (ml/100 g per min)
		pH	pH*T2		
Interaction with scan-order[a]	No	Yes	Yes	No	No
Global	2.2 (-1.1 to 5.4)	1.9 (-4.5 to 8.1)	-5.7 (-10.7 to -5.7)	0.04 (-2.9 to 3.1)	-3.7 (-6.0 to -1.3) [b]
Regional:					
Frontal lobe	3.5 (1.1 to 5.7) [b]	7.0 (1.7 to 11.0) [b]	-9.7 (-12.7 to -5.3) [c]	-2.1 (-3.3 to -0.7) [b]	-4.6 (-6.9 to -2.1) [b]
Parietal lobe	4.2 (2.0 to 6.2) [c]	2.7 (-2.0 to 7.0)	-7.1 (-10.0 to -3.8) [c]	-2.5 (-3.9 to -1.0) [b]	-4.7 (-7.5 to -1.6) [b]
Temporal lobe	3.1 (0.9 to 5.3) [b]	1.5 (-2.8 to 5.7)	-5.8 (-8.8 to -2.8) [c]	-1.7 (-2.8 to -0.6) [b]	-4.6 (-6.9 to -2.2) [b]
Occipital lobe	4.4 (0.9 to 7.7) [d]	-1.6 (-8.4 to 4.5)	-6.6 (-11.6 to -1.6) [d]	-3.0 (-4.9 to -0.9) [b]	-5.0 (-7.8 to -1.9) [b]
Cerebellum	3.9 (0.5 to 7.2) [d]	2.5 (-3.9 to 8.6)	-8.4 (-13.4 to -3.7) [b]	-2.5 (-3.6 to -1.3) [c]	-4.7 (-8.2 to -1.0) [d]
Thalamus	5.2 (0.9 to 9.3) [d]	1.1 (-7.4 to 9.3)	-10.0 (-14.4 to -1.5) [d]	-1.0 (-4.8 to 3.1)	-5.8 (-10.0 to -1.5) [d]

Associations were studied using linear mixed effects models including a random intercept and slope. The estimated effect (95% CI) of the individual characteristics on CBF is presented. Of the *a priori* selected factors, the analysis of MAP and CBF was limited by insufficient power due to missing values and patient variation, and is considered inconclusive. Hematocrit was associated with CBF only in one VOI.

[a]No interaction with scan-order means that the effect of pCO_2, temperature, and UF volume on CBF is similar at T1, T2, and T3. The pH model could be interpreted by adding the effect of the single term 'pH' and of the interaction term 'pH*T2', yielding a net negative effect of pH on CBF at T2 as compared with T1.

[b] *P*<0.01; [c] *P*<0.001; [d] *P*< 0.05.

Associations of Cognitive Function and Structural Markers of Brain Lesions with CBF

No significant correlation between cognitive function, or structural markers of brain lesions (*i.e.* the Fazekas score indicating severity of white matter lesions, and the presence of microbleeds) and baseline global or regional CBF was found (Supplemental Table 2).

Additionally, we tested the associations between cognitive function and structural markers of brain lesions with CBF using LMM, thereby including all CBF measurements.

These analyses should be considered as hypothesis generating because of the relatively small sample size. Cognitive function and structural markers of brain lesions were not associated with global CBF (Supplemental Table 3). For regional CBF, a better executive function according to the Z-converted Trail Making Test B (TMT-B) and according to the TMT B/A ratio was associated with higher CBF at T2 as compared with T1 in several brain regions (Supplemental Table 3). A higher Fazekas score, indicating more severe white matter lesions, was associated with higher CBF in most regions at T2 as compared with T1. The presence of microbleeds was associated with higher CBF of the temporal lobe and cerebellum at T2 as compared with T1.

Adverse Event

One patient (identity 115) lost consciousness due to dialysis-induced hypotension shortly after the third scan. CBF decreased from 30.4 predialysis to 24.2 mL/100 g per minute (-20%) at T3 shortly before he lost consciousness. This patient made a full recovery without sequelae. None of the other patients experienced intradialytic hypotension (IDH, i.*e*., SBP<100 mmHg, or IDH symptoms), or received any intervention for IDH during the HD study session.

Sensitivity Analyses

Because CBF changes in the left and right hemispheres did not differ significantly, the hemispheres were merged for the aforementioned VOIs analyses. The results were basically identical when both hemispheres were analyzed separately (Supplemental Table 4).

In the analysis with T2 as the reference point for CBF change, global and regional CBF declined significantly between T2 and T3 as well (Table 3). Global and regional CBF did not differ significantly between T1 and T2.

The HD-induced change in regional perfusion of the gray matter was analyzed separately as opposed to the combined gray and white matter perfusion of these regions. In all VOIs, the decline in gray matter perfusion was similar to or even greater than the sum of gray and white matter (Supplemental Table 5).

DISCUSSION

The main finding of our study is that CBF declined by 10% ±15% during a conventional HD session in elderly patients on maintenance HD. The decline in CBF was similar for the various individual brain regions that were studied and therefore, most likely, affected both the anterior (*i.e.* the internal carotid arteries) and posterior (*i.e.* the vertebral and basilar arteries) circulation. The decline in CBF (-20%) was symptomatic in one patient.

HD treatment-related factors that might explain the intradialytic CBF decline were a higher tympanic temperature, a greater UF volume and UF rate, and a higher pH.

This study is new insofar as that CBF was quantitatively measured early and late during HD using a gold-standard technique, *i.e.* with [^{15}O]H_2O PET-CT scans. Previous studies estimating CBF during HD reported contradictory results and were limited by the use of the transcranial Doppler technique, which measures CBF velocity, and represents CBF only if the diameter of the insonated vessel remains constant during HD. [23-28]

Under normal physiologic conditions, CBF depends on cerebral perfusion pressure and cerebrovascular resistance. Hypothetically, CBF is kept relatively constant by cerebral autoregulation, a complex interplay of metabolic, myogenic, and neurogenic mechanisms. Whether HD affects these mechanisms due to the inherent hemodynamic stress and metabolic changes, is currently unknown. However, this study suggests that several HD treatment-related mechanisms might be involved in the intradialytic decline of CBF. First, cerebral perfusion pressure, defined as the difference between MAP and intracranial pressure, will depend largely on the MAP during HD. In this study, MAP decreased significantly between T2 and T3 but, unfortunately, the analyses of the association between MAP and CBF were inconclusive. Interestingly, a larger UF volume and rate, which may indicate greater hemodynamic stress, were associated with lower CBF. Second, cerebrovascular resistance might be modulated by intradialytic changes in metabolic factors, blood viscosity, and body temperature. pCO_2, which was positively associated with CBF, remained constant during HD and did not explain the HD-induced CBF decline. A higher pH was associated with lower CBF only shortly after the start of HD, as compared with before the start of HD, but not at the end of HD. Hematocrit reflects blood viscosity, and an increase in hematocrit was reported to reduce CBF. [29] In this study, the rise in hematocrit was very small and is unlikely to explain the decline in CBF. Finally, a higher tympanic temperature was associated with lower CBF, which is in accordance with a previous trial on dialysate cooling by Eldehni *et al.* [15] These authors reported that lower dialysate temperature, which is thought to improve vascular resistance, [30] led to improved hemodynamic stability and prevented the development of white matter lesions in incident patients with incident HD compared with the use of a dialysate temperature of 37.0°C. [15, 31] Notably, in this study we used a relatively low dialysate temperature (36.5°C) and kept the room temperature stable at 20°C. Even then, CBF declined significantly.

An important question is whether repetitive HD-induced CBF declines are causally related to ischemic brain lesions and cognitive decline. To our knowledge, no data are available on clinical effects of a similar intervention-related CBF decline. Generally, the CBF threshold for ischemia is considered as <10 mL/100 g per minute, and <20 mL/100 g per minute for the penumbra that surrounds an ischemic event, indicating severely ischemic but still viable brain tissue. [32] In this study, these absolute CBF thresholds were

not reached, because the lowest individual CBF level was 24.4 mL/100 g per minute at T3. However, whether CBF reductions lead to ischemia also depends on the duration of the CBF reduction, blood oxygenation, the efficacy of oxygen extraction, and capillary (dys)function. [33] The importance of oxygenation was underscored by a recent study that showed that a relative drop of 15% in cerebral oxygenation during HD, defined as cerebral ischemia, was associated with decreased executive cognitive function at 12 months.[34] Additionally, cerebral oxygenation was reported to be lower in patients receiving HD compared with patients receiving peritoneal dialysis, and with controls. [35-37] Moreover, oxygen extraction fraction was lower in patients receiving HD compared with controls.[38] An underlying reason for the lower oxygen extraction fraction might be the concept of capillary dysfunction, which was recently proposed as a source of stroke-like symptoms and cognitive decline. [33] In capillary dysfunction, changes in capillary flow patterns can limit the oxygen extraction in tissue, thereby making tissue hypoxia possible even in the presence of adequate cerebral blood supply. [33] Thus, a CBF decline together with low cerebral oxygenation, and low oxygenation extraction might put the brain at risk for ischemia at a relatively higher CBF in patients receiving HD compared with nondialysis patients. Interestingly, endothelial injury and dysfunction, which is a common feature in patients receiving HD, [39, 40] is considered an important source of capillary dysfunction. [33] In this study HD-induced endothelial dysfunction likely occurred because plasma levels of myeloperoxidase and pentraxin 3 rose significantly during HD. [41-43]

A limitation of our study is that we included a relatively small number of subjects due to the practical challenges of performing intradialytic PET-CT scans, especially in elderly. Nevertheless, [^{15}O]H_2O PET-CT is the gold standard to measure CBF and this is the first study that quantitatively studied CBF during HD. Another limitation is that we included only elderly patients, with a relatively long median dialysis vintage, thereby limiting generalizability of our findings to the general dialysis population. In the light of the small sample size, our findings with respect to the secondary objective of this study, *i.e.* associations of HD treatment-related factors with CBF, should be considered with caution. Future studies with a larger cohort of patients are needed to evaluate the intradialytic course of CBF in relation to simultaneous changes of MAP, pH, temperature, and hematocrit, and UF volume and rate, because the identification of HD treatment-related factors involved in CBF decline might help guide the design of new HD protocols that minimize cerebrovascular stress. Additionally, the longitudinal association between HD-induced CBF declines and cognitive function needs further attention.

In conclusion, conventional HD induces a significant reduction in global and regional CBF in elderly HD patients. Repetitive intradialytic decreases in CBF may be one of the mechanisms by which HD induces cerebral ischemic injury.

SIGNIFICANCE STATEMENT

Evidence increases that the hemodialysis procedure might induce brain injury. The transition to dialysis has been associated with a significant loss of cognitive function. Furthermore, cerebral ischemic injury increased after hemodialysis initiation and lowering the dialysate temperature attenuated the progression of white matter lesions in the brain. However, the mechanism by which hemodialysis could contribute to brain injury is unknown. This study demonstrates that hemodialysis induces a significant reduction in brain perfusion. This reduction might be a mechanism underlying the ischemic brain injury. A higher pH, body temperature, and ultrafiltration volume and rate were associated with lower brain perfusion, and might form a point of departure for further research to develop hemodialysis protocols that minimize or prevent cerebrovascular stress.

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SUPPLEMENTAL MATERIAL

FULL METHODS

Patients and Study Design

This study was performed according to the principles of the Declaration of Helsinki and was approved by the Medical Ethical Committee of the University Medical Center Groningen, and registered at clinicaltrials.gov (NCT02272985). All patients gave written informed consent. The study was performed between March and November of 2015.

Patients receiving HD aged ≥65 years from our department with an arteriovenous fistula without significant recirculation were eligible for this study. Patients were studied during a regular dialysis session after the longest interdialytic interval (Monday or Tuesday). Patient characteristics were assessed at study entry and retrieved from the patients' medical history. Height was measured before, and weight before and after the PET-HD session. BP, heart rate, and tympanic temperature were measured before every PET-CT scan and every 30 minutes during the HD study session.

Hypertension was defined as predialysis systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or the use of anti-hypertensive drugs. UF rate was expressed in mL/h per kg body weight by dividing ultrafiltration volume by dialysis session length and postdialysis target weight. Equilibrated Kt/V was calculated according to the second-generation logarithmic Daugirdas. [1]

Based on the highly sensitive technique of $[^{15}O]H_2O$ and based on former studies that mainly used TCD in which the number of HD patients varied between 12 and 27 [2-7], we expected that a total of 14 patients would be sufficient, and aimed to include 14 patients. Additional inclusion criteria were a hemoglobin level between 6.2 and 8 mmol/L since at least 1 month before inclusion, because hemoglobin levels are associated with CBF. Patients with a history of dementia, hydrocephalus, cerebrovascular accident, raised intracranial pressure, end-stage liver disease, actively treated cancer, a known significant (>70%) internal carotid artery or major intracranial vessel stenosis, and patients with a contra-indication for MRI were excluded. After study-inclusion, routine duplex evaluation was performed to exclude subjects with an asymptomatic internal carotid artery stenosis of more than 70% or major intracranial vessel stenosis, because this may interfere with the interpretation of CBF (change).

HD Study Session

All HD study sessions were performed in the afternoon in the PET-camera room. The ambient temperature of the room was kept constant at 20˚C, excluding an effect of outside temperature on cardiovascular stability during study sessions. After the first PET

scan (T1), patients started dialysis still being in a horizontal position in the PET-camera. After the second PET scan (T2), which was performed within 30 minutes after the start of HD, patients were transferred to a hospital bed adjacent to the PET-camera to continue dialysis in a 30-45-degree supine position. Approximately 30 minutes before the start of the third PET scan (T3), which was performed in the final hour of the HD session, patients were transferred back to the PET.

A low-dose brain computed tomography was made before the first and third PET-scan to correct for attenuation of the PET data. A bolus injection of $[^{15}O]H_2O$ was administered intravenously at a constant rate through an indwelling peripheral venous catheter in the non-dialysis access arm. The injected dose of $[^{15}O]H_2O$ was 500 MBq per scan, with a total dose of 1500 MBq per patient for the whole study. During each PET-scan, arterial blood was sampled continuously from the dialysis line by a dedicated programmable blood-sampler to obtain the course of the radioactivity concentration in the blood during 5 minutes following the injection of $[^{15}O]H_2O$. To perform laboratory measurements, arterial blood was sampled from the arterial dialysis line just before each PET-scan.

Dialysis Settings

All patients were on bicarbonate dialysis with a low-flux polysulfone hollow-fiber dialyzer (F8; Fresenius Medical Care, Bad Homburg, Germany). Blood flow and dialysate flow rates were 200-300 and 500 mL/min, respectively. Dialysate temperature was 36.5°C in all patients. We used constant UF rate and dialysate conductivity. Dialysate composition was sodium 139 mmol/L, potassium 1.0 or 2.0 mmol/L depending on the prevailing plasma potassium, calcium 1.5 mmol/L, magnesium 0.5 mmol/L, chloride 108 mmol/L, bicarbonate 34 mmol/L, acetate 3.0 mmol/L, and glucose 1.0 g/L. The water for hemodialysis complied with the requirements of the European Pharmacopoeia (<100 colony-forming units/mL; <0.25 endotoxin units/mL).

PET Data Acquisition

For the $[^{15}O]H_2O$ PET-CT scans a Siemens Biograph 64-mCT (Siemens Medical Systems, TN) that acquires 109 planes over a total axial length of 216 mm was used. $[^{15}O]H_2O$ was produced by conversion of the $[^{15}O]O_2$ using a new designed IBA chemistry module (IBA RadioPharma Solutions, Belgium) and placed in a shielded class A foam hood located in the Good Manufacturing Practice laboratory. During preparation, ^{15}O gas flew from the cyclotron into the IBA chemistry module and was mixed with hydrogen gas and passed through a palladium column. The produced $[^{15}O]H_2O$ was collected in a sterile 0.9% saline solution to obtain a final product suitable for patient administration. The method was validated and met all pharmacopeia specifications. The practical production yield of ^{15}O labeled water using this method ranged between 1300-1700 MBq measured in the syringe. $[^{15}O]H_2O$ was produced one floor below the PET location facilitating rapid

transport to the PET-camera room, which is important since its half-life is short (T½ 2.03 min).

First, a low-dose computed tomography scan was performed for attenuation and scatter correction. The dynamic PET acquisition (310 seconds) was started, followed after 10 seconds by an intravenous bolus injection of [^{15}O]H_2O. In total, the duration of every PET-CT scan was 5 minutes, which was uniform across all time points and all patients. Head movement was minimized with a head-restraining band. For CBF quantification, the arterial input function was obtained from arterial blood radioactivity, which was continuously monitored with an automated sampling system (Veenstra Instruments, Joure, the Netherlands). One extra blood sample was collected at 393±32 seconds after tracer injection to determine the amount of radioactivity in the blood using a γ-counter (Wizard2, Perkin Elmer, Waltham).

Three of the 36 scans could not be analyzed due to a technical problem with the automated sampling system during the measurement (patient identity 106 [T1], patient identity 107 [T2], patient- identity 102 [T3]).

MRI Data Acquisition

MRI was performed using a 1.5T whole body system (Aera, Siemens, Erlangen, Germany) on a non-dialysis day. The study MRI was performed median 3 days (range, -72 to +3 days) after the HD study session. The scan protocol (total scan time 30 minutes) included T1-weighted, T2-weighted, three-dimensional fluid-attenuated inversion recovery, diffusion-weighted imaging, susceptibility weighted imaging, and two-dimensional phase contrast sequences. No intravenous contrast was used. A neuroradiologist (PJvL) assessed white matter hyperintensities, and cortical atrophy, using the Fazekas scale and the global cortical atrophy scale, respectively. [8, 9] Microbleeds were scored on the susceptibility weighted imaging sequence.

Image Reconstruction and Preprocessing

Image processing and pharmacokinetic analysis were performed with PMOD 3.8 software (PMOD Technologies Ltd., Zurich, Switzerland). The average image (time-weighted) was used for rigid matching registration of the individual PET to the individual MRI.

The PET list-mode data were reconstructed using the 3D OSEM algorithm (3 iterations and 24 subsets), point spread function correction and time-of-flight, and reconstructed to 28 dynamic frames (1×10 sec, 12×5 sec, 6×10sec, and 9×20 sec). Data were corrected for attenuation, scatter and radioactivity decay. This resulted in images with a matrix of 400 × 400 × 111 of 2 mm voxels, smoothed with a 2 mm filter at full width at half maximum.

We used the 3D T2-FLAIR images for the registration process, because the 3D acquisition of the T1-weighted sequence was not available. Furthermore, several patients had

marked brain atrophy and white matter lesions. Therefore, we used the population-based gray matter/white matter (GM/WM) maps to segment the cortical tissue, instead of using the subject probability maps. This means that the cortical volumes of interest (VOIs) are slightly larger than when the individual maps for the subject are used. Since we did the modeling in the subject brain space (no deformations to adjust to the atlas) and the VOIs were based on the population-based GM/WM probabilities, the effect of the atrophy and lesions is expected to be minimal.

Predefined VOIs were transformed into the individual space, based on the Hammers atlas and limited to the gray matter tissue in the cortical regions (>30% gray matter probability based on standard probability). [10] After spatial registration, pharmacokinetic modeling was applied to the dynamic PET images to calculate the CBF, based on the implementation of the 1-tissue compartment model developed by E. Meyer. [11] Delay of the arterial input function and dispersion in the model were first calculated for the whole brain, and then these resulting values were fixed for the brain regions.

Neuropsychological Tests

A neuropsychological assessment battery was performed to characterize the study population and included all major cognitive domains. The battery included the Mini Mental State Examination (MMSE; measuring global cognitive function), digit span (measuring attention), Trail Making Test A and B (TMT A and B; measuring attention, executive function including set shifting, and motor speed), clock drawing (measuring executive function, visuospatial skills), verbal fluency (measuring executive function, language), Dutch version of the RAVLT (measuring verbal memory, immediate and delayed recall, and recognition) and the Hospital Anxiety and Depression Scale (HADS) for identifying depression and anxiety symptoms. The order of the tests was fixed and cognitive testing was performed on a non-dialysis day. It took approximately 45-60 min per subject to complete the tests. The neuropsychological assessment was performed median 95 days (range, -196 to -33 days) before the PET-HD session.

Statistical Analyses

Intradialytic changes in levels of the HD-related characteristics were studied using repeated measures ANOVA (with a Greenhouse-Geisser correction in case of non-sphericity), with a Bonferroni correction.

For the primary study objective, global and regional CBF changes were analyzed by LMM, which allowed for individual random intercepts and slopes of CBF over time. The random slopes were on the basis of the actual scan times per patient. Relative CBF change was calculated as the mean of the individual percentual change between T1 and T3 using descriptive statistics, and is reported as mean±SD (%).

For the secondary study objective, associations of HD treatment-related factors, which might potentially explain CBF change, with CBF were studied. Those factors included MAP, pCO_2, pH, tympanic temperature, hematocrit, and UF volume and were selected based on literature. [2-4, 12-16] The factors were studied univariately using LMM, checking the significance of interactions with scan-order. Because UF volume was associated with CBF, the association between UF rate and CBF was evaluated as well.

In additional analyses, associations of cognitive test scores and structural brain characteristics with CBF were explored. To this end, we first tested correlations with baseline CBF using Pearson or Spearman correlation, if appropriate. Subsequently, we studied the associations including all CBF measurements in a LMM. For these analyses, the cognitive test scores were converted to Z scores.

Several sensitivity analyses were performed. First, regional CBF change was also calculated for the left and right hemisphere separately. Second, in order to eliminate a possible effect of HD on the arterial sampling from the arteriovenous fistula, CBF change between T2 and T3 was calculated. Third, CBF change in only the gray matter of each VOI was studied instead of the sum of gray and white matter of the corresponding region.

Two-sided $P<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS, version 23 (SPSS Inc., IBM company), GraphPad Prism version 5.0 (GraphPad Software, San Diego), and R version 3.4.0 (R Core Team, 2017).

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Supplementary Table S1 Intradialytic BP trajectories of the individual study participants

	Before start HD		Nadir during HD		Interval between the start of HD and nadir
Study identity	SBP (mmHg)	MAP (mmHg)	SBP (mmHg)	MAP (mmHg)	(minutes)
101	166	112	169	122	145
102	164	103	146	105	163
103	166	N.A.	N.A.	N.A.	N.A.
105	151	102	105	90	70
106	107	77	108	77	158
107	136	96	105	84	6
108	191	113	158	102	152
109	143	102	131	100	167
110	130	88	108	67	260
112	156	99	158	97	138
114	144	102	106	79	253
115	173	116	140 [a]	103 [a]	132[#]

HD, hemodialysis; MAP, mean arterial pressure; N.A., not available; SBP, systolic blood pressure.

[a] This patient (identity 115) lost consciousness due to dialysis-induced hypotension shortly after the third scan (T3). BP was not measured during this event; the next measured BP after regaining consciousness was 155/90. Thus, the nadir presented in this table is not the nadir at the moment of the dialysis-hypotension episode.

Supplementary Table S2 Correlations of cognitive function and structural markers of brain lesions with baseline CBF

	Global CBF		Regional CBF											
			Frontal lobe		Parietal lobe		Temporal lobe		Occipital lobe		Cerebellum		Thalamus	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Cognitive tests:														
zMMSE	-0.03	0.9	-0.05	0.9	-0.04	0.9	0.05	0.9	0.09	0.8	0.05	0.9	0.02	1.0
zDigit Span forward	0.20	0.6	0.20	0.5	0.16	0.7	0.39	0.2	0.39	0.2	0.28	0.4	0.23	0.5
zDigit Span backward	-0.28	0.4	-0.22	0.5	-0.28	0.4	-0.08	0.8	-0.30	0.4	-0.43	0.2	-0.06	0.9
zRAVLT delayed recall	0.09	0.8	0.04	0.9	0.05	0.9	0.12	0.7	-0.02	1.0	0.01	1.0	0.20	0.6
zTMT A	0.06	0.9	0.01	0.9	-0.02	0.9	-0.01	1.0	0.10	0.8	0.37	0.3	-0.10	0.8
zTMT B	0.16	0.6	0.14	0.7	0.16	0.7	0.04	0.9	0.29	0.4	0.17	0.6	-0.28	0.4
TMT B/A ratio	0.11	0.8	0.08	0.8	0.08	0.8	0.06	0.9	0.23	0.5	0.12	0.7	-0.16	0.7
zVerbal fluency	-0.17	0.6	-0.24	0.5	-0.23	0.5	-0.04	0.5	-0.16	0.6	-0.14	0.7	-0.19	0.6
zClock drawing	-0.14	0.7	-0.10	0.8	-0.08	0.8	-0.11	0.8	-0.13	0.7	-0.28	0.4	0.12	0.7
Structural markers of brain lesions:														
Microbleeds	-0.19	0.6	-0.13	0.7	-0.09	0.8	-0.22	0.5	-0.36	0.3	-0.32	0.4	0.03	0.9
Fazekas score	-0.13	0.7	0.01	1.0	0	1.0	-0.18	0.6	-0.37	0.3	-0.38	0.3	0.12	0.7

Correlations were calculated using Pearson or Spearman correlation, if appropriate. The cognitive tests scores were converted to Z scores for the analyses. For regional CBF, the mean CBF of the left and right hemisphere was used for the analyses.

MMSE, Mini Mental State Examination; r, correlation coefficient; RAVLT, Ray Auditory Verbal Learning Test; *P*, *P* value indicating significance level; TMT, trail making test.

Supplementary Table S3 Associations of cognitive function and structural markers of brain lesions with CBF[1]

	zTMT B [a]		TMT B/A ratio [b]		Fazekas score [c]		Microbleeds	
Region	Estimated effect on CBF (mL/100 g per min)		Estimated effect on CBF (mL/100 g per min)		Estimated effect on CBF (mL/100 g per min)		Estimated effect on CBF (mL/100 g per min)	
	zTMT B	zTMT B*T2	TMT B/A	TMT B/A*T2	Fazekas	Fazekas*T2	MB	MB*T2
Interaction with scan-order[d]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Global	NS	NS	0.6 (-2.9 to 4.1)	-1.9 (-4.1 to 0.2)	-1.0 (-4.6 to 2.5)	2.0 (-0.1 to 3.8)	NS	NS
Regional								
Frontal	NS	NS	0.6 (-4.3 to 5.5)	-2.2 (-3.9 to 0.6) [e]	-0.6 (-5.0 to 3.7)	1.3 (-0.1 to 2.7)	NS	NS
Parietal	0.5 (-4.2 to 5.2)	-1.5 (-2.9 to -0.1) [e]	0.9 (-4.4 to 6.1)	-2.5 (-4.1 to -0.9) [f]	-0.5 (-5.1 to 3.9)	1.7 (0.4 to 3.0) [e]	NS	NS
Temporal	NS	NS	0.5 (-3.5 to 4.4)	-2.2 (-3.6 to -0.8) [f]	-1.3 (-4.9 to 2.3)	2.5 (1.4 to 3.5) [g]	1.4 (-7.5 to -4.9)	2.5 (0.2 to 4.8) [e]
Occipital	NS	NS	1.5 (-2.8 to 5.7)	-3.0 (-5.3 to -0.7) [e]	-2.4 (-6.4 to 1.6)	4.1 (2.5 to 5.7) [g]	NS	NS
Cerebellum	1.9 (-4.1 to 0.2)	-2.0 (-3.7 to -0.3) [e]	0.9 (-3.9 to 5.7)	-3.0 (-4.9 to -1.0) [f]	-3.2 (-7.8 to 1.5)	4.2 (2.7 to 5.7) [g]	-3.2 (-11.0 to -4.9)	5.4 (-1.5 to 11.9) [e]
Thalamus	NS	NS	-1.1 (-6.1 to 3.9)	-2.9 (-5.4 to -0.3) [e]	0.5 (-4.6 to 5.6)	3.5 (1.3 to 5.8) [f]	NS	NS

Associations were studied using linear mixed effects models including a random intercept and slope. The estimated effect (95%CI) of the individual characteristics on CBF is presented. The zMMSE, zDigit span backwards and forwards, zFluency, zTMT A, zRAVLT delayed recall, and zClock drawing test scores were not associated with CBF. MB, microbleeds; NS, not significant; TMT, trail making test.

[a,b] A higher zTMT B score or TMT B/A ratio indicates worse executive function.

[c] The Fazekas score was entered to the model as a continuous covariate.

[d] The interaction models could be interpreted by adding the effect of the single term and the interaction term, e.g. yielding an association of a higher zTMT B score with a lower CBF at T2 as compared with T1.

[e] $P<0.05$; [f] $P<0.01$; [g] $P<0.001$.

1 Note to the reader: the associations presented in this table can only be viewed as hypothesis generating since the relatively small sample size militates against definitive conclusions. The cognitive test results were not corrected for age and education due to the small sample size. Furthermore, we were not able to analyze the Fazekas score (which is an ordinal variable including 4 categories) also as a categorical variable due to the small sample size

Supplementary Table S4 Intradialytic changes in regional CBF, specified per hemisphere

	Before start HD	After start HD	At the end of HD
Brain region	T1	T2	T3
Frontal lobe L	39.9 ± 6.9	38.6 ± 5.5	34.8 ± 4.9
Frontal lobe R	40.4 ± 6.9	39.2 ± 5.7	35.2 ± 4.6
Parietal lobe L	37.1 ± 7.1	36.0 ± 6.3	32.3 ± 5.3
Parietal lobe R	37.7 ± 6.9	36.7 ± 6.2	32.9 ± 4.9
Temporal lobe L	35.5 ± 5.3	35.4 ± 5.6	31.5 ± 4.6
Temporal lobe R	36.2 ± 4.9	36.0 ± 5.7	32.1 ± 4.8
Occipital lobe L	41.4 ± 5.5	41.3 ± 7.3	37.3 ± 5.4
Occipital lobe R	42.3 ± 4.8	41.9 ± 6.7	38.1 ± 6.0
Cerebellum L	43.3 ± 6.7	44.7 ± 7.3	38.4 ± 5.8
Cerebellum R	43.2 ± 7.0	44.9 ± 7.6	38.5 ± 6.5
Thalamus L	47.2 ± 7.8	48.1 ± 9.1	41.7 ± 9.0
Thalamus R	47.3 ± 7.3	48.2 ± 8.2	41.7 ± 7.9

CBF data (mL/100 g per minute) are presented as unadjusted means ± SD. [a] Scan 2 (T2) and 3 (T3) were performed at mean 21 and 209 minutes after start of HD (T1), respectively. L, left; R, right.

Supplementary Table S5 Intradialytic changes in regional CBF of the gray matter only

	Before start HD	After start HD	At the end of HD	Dialysis treatment effect	
	T1	T2	T3	T1 vs. T3	T2 vs. T3
Frontal gray matter	44.4 ± 7.7	43.1 ± 6.4	38.5 ± 5.2	-5.8 (-10.8 to -0.9) [a]	-4.7 (-8.9 to -0.5) [a]
Parietal gray matter	42.2 ± 8.1	41.0 ± 7.5	36.7 ± 5.7	-5.6 (-10.1 to -1.0) [a]	-4.8 (-8.7 to -0.9) [a]
Temporal gray matter	38.7 ± 5.5	38.4 ± 6.2	34.2 ± 5.1	-4.5 (-8.2 to -0.7) [a]	-4.4 (7.6 to -1.2) [b]
Occipital gray matter	43.8 ± 5.4	43.5 ± 7.5	39.4 ± 5.9	-4.6 (-9.0 to -0.3) [a]	-4.8 (-8.7 to -1.0) [b]
Cerebellum gray matter	43.9 ± 6.9	45.4 ± 7.5	38.9 ± 6.3	-5.1 (-9.3 to -0.8) [a]	-6.4 (-10.2 to -2.7) [c]

CBF data (mL/100 g per minute) are presented as unadjusted mean ± SD. Scan 2 (T2) and 3 (T3) were performed at mean 21 and 208 minutes after start of HD (T1), respectively. Dialysis treatment effects are obtained from linear mixed effects models including a random intercept and slope, and presented as mean difference (95% CI). [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$.